Bonneville Power Administration
Fish and Wildlife Program FY99 Proposal

Section 1. General administrative information

Assessing Genetic Variation Among Columbia Basin White Sturgeon Populations

Bonneville project number, if an ongoing project 9084

Business name of agency, institution or organization requesting funding
University of Idaho

Business acronym (if appropriate)

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Subcontractors.

Organization	Mailing Address	City, ST Zip	Contact Name

NPPC Program Measure Number(s) which this project addresses.
10.4, 10.4A.1 through 10.4A5, 10.6C, 10.6C.1, 10.8B15, 10.8B16.

NMFS Biological Opinion Number(s) which this project addresses.
Kootenai River White Sturgeon Aquaculture Program (DOE-EA-1169), USFWS 1994 Biological Opinion on the 1994-1998 Federal Columbia River Power Operation Assessment, USFWS, Biological Opinion 1995 1-4-95-F-003 (Kootenai River White Sturgeon)

Other planning document references.
Zone 6 Plan Reference of ODFW, WDFW, and CRITFC, 86-50 SOW Task 1.2

Subbasin.
Columbia, Snake, and Kootenai River Basins

Short description.
Genetic variation and stock structure among white sturgeon populations in the Columbia Basin based on analysis of mitochondrial and nuclear DNA.

Section 2. Key words

Mark	Programmatic Categories	Mark	Activities	Mark	Project Types
	Anadromous fish		Construction		Watershed
X	Resident fish		O & M	X	Biodiversity/genetics
	Wildlife		Production		Population dynamics
	Oceans/estuaries	X	Research		Ecosystems
	Climate		Monitoring/eval.		Flow/survival
	Other		Resource mgmt		Fish disease
			Planning/admin.		Supplementation
			Enforcement		Wildlife habitat en-
			Acquisitions		hancement/restoration

Other keywords.
DNA, white sturgeon, stock identification, genetic variation, evolutionary significant units

Section 3. Relationships to other Bonneville projects

Project #	Project title/description	Nature of relationship
86-50	White sturgeon mitigation and restoration in the Columbia and Snake Rivers	This project will provide genetic information for management. The 86-50 project provides tissue samples for this project.
94-49	Kootenai River ecosystem and fisheries improvement study	This project will provide genetic information on an endangered population. The 94-49 project provides tissue samples for this project.
88-64	Kootenai River fisheries studies	This project will provide genetic information on an endangered population. The 88-64 project provides tissue samples for this project.
88-65	Kootenai river fisheries	This project will provide genetic

	investigations	information on an endangered population. The 88-65 project provides tissue samples for this project.

Section 4. Objectives, tasks and schedules

Objectives and tasks

Obj 1,2,3	Objective	Task a,b,c	Task
1	Preliminary assessment of genetic variation among Columbia Basin white sturgeon	a	Mitochondrial D-loop length variation will be described and compared pair-wise between geographically proximate samples and amon geographically separated samples.
2	Assessment of mitochondrial sequence divergence among Columbia Basin white sturgeon.	a	Sequence divergence of a non-repetitive portion of the D-loop will be compared among samples from each location listed in the first objective.
3	Assessment of nuclear genetic variation among Columbia Basin white sturgeon populations	a	Eight nucleotide primer pairs for microsatellite loci will be used to examine nuclear genetic variation in the same populations listed previously.

Objective schedules and costs

Objective #	Start Date mm/yyyy	End Date mm/yyyy	Cost %
1	1/1999	12/1999	18.00%
2	6/1999	5/2000	32.00%
3	6/1999	12/2001	50.00%
			TOTAL 100.00%

Schedule constraints.

Difficult sequencing or equipment breakdown for automated sequencing may lengthen the time required to complete Objective #2 and some of Objective #3. Projections for completion are: Objective #1; 1999, Objective #2; 2000, Objective #3; 2001

Completion date.
2001

Section 5. Budget

FY99 budget by line item

Item	Note	FY99
Personnel	M. Powell, 6 mo. @ 24.00/hr. (1040 h) P. Anders, 12 mo. @ 16.90/hr. (2080 h)	60112
Fringe benefits	28.5% for both	17132
Supplies, materials, non-expendable property	chemicals, pipet tips, tubes, gloves etc.	20000
Operations & maintenance	Equipment service and calibration, UPS shipping, Federal Express, long distance calls/faxing	2000
Capital acquisitions or improvements (e.g. land, buildings, major equip.)	1 Power Macintosh Computer and Perkin Elmer ABI Genotyper Software	11000
PIT tags	# of tags: 0	$0
Travel	1 professional meeting (AFS), 2 people, travel between laboratories	$1,500
Indirect costs	off campus indirect cost @ 25.8%	25992
Subcontracts	0	$0
Other	0	$0
TOTAL		$137,736

Outyear costs

Outyear costs	FY2000	FY01	FY02	FY03
Total budget	$148,000	$152,000		
O&M as % of total	1.50%	1.50%		

Section 6. Abstract

The genetic relationships of white sturgeon (*Acipenser transmontanus*) populations within the Columbia Basin remain unclear. To date, there has been no comprehensive genetic assessment of sturgeon populations in the Columbia, Snake and Kootenai River systems using any analytical method. The objective of this project is to

assess inter- and intrapopulational genetic variation among white sturgeon in the Columbia, Snake, and Kootenai River Basins. This project will employ both nuclear and mitochondrial DNA analyses to test the null hypothesis; white sturgeon populations in the Columbia Basin represent (a) a single gene pool, and (b) one ESU of the species. This project is essential to most aspects of the Columbia Basin Fish and Wildlife Program and directly addresses section 10.4A "Study and evaluate Sturgeon Populations." For example, falsification of this hypothesis will critically effect the management of white sturgeon populations within these watersheds. Divergent populations will have to be considered for management as significant components of overall white sturgeon diversity, possibly requiring additional measures, such as supplementation, to ensure their long term stability or recovery. It is expected that this comprehensive genetic assessment will require three years to complete. The results will be reviewed and assessed by those currently involved in BPA funded white sturgeon research and recovery throughout the Columbia Basin.

Section 7. Project description

a. Technical and/or scientific background.

In the Columbia River Basin, white sturgeon constitute an ecologically, economically, and in some cases, a culturally and spiritually important resource. Although the viability of many white sturgeon populations in the Basin is currently unknown, some are declining and one, the Kootenai River population, is listed as endangered and considered at risk of extinction (FR 59: 171). Accordingly, state, federal, tribal, private, and provincial agencies are considering or have initiated protection and restoration measures throughout the Basin to maintain and restore white sturgeon population numbers. While demographic and environmental conditions affect short-term survival of white sturgeon populations, their long-term survival may be adversly affected by a loss of genetic variability (see Avise, 1994 for a general review). Since long-term viability and persistence of fish populations are largely determined by the size and genetic variation within the effective population, research addressing short-term population dynamics and environmental conditions may be inadequate to ensure long-term survival of white sturgeon populations in the Columbia River Basin.

Previous examinations of genetic variation among regional white sturgeon populations using protein electrophoresis conducted at the University of Idaho have demonstrated a loss of genetic variation in the Kootenai River population relative to downstream Columbia River Basin populations (Bartley et al., 1985; Setter and Brannon, 1992). However, the level of genetic variation or the degree which conspecifics in the Columbia and Snake Rivers form genetically distinct populations or evolutionary significant units (ESUs) remains unknown (see Setter and Brannon 1992 to review results of allozyme analysis on white sturgeon populations). To further address questions regarding the genetics and conservation of white sturgeon basin-wide, the Aquaculture Research Institute (ARI) at the University of Idaho initiated a long-term research strategy, as a collaborative project with agencies and Native American Tribes. Currently, white sturgeon are considered by some to exist potentially as genetically distinct populations

separated by hydroelectric dams throughout the Columbia Basin. However considerable white strugeon migration (and hence gene flow) has been documented among many Columbia River reserviors. For example, over 3,000 white strugeon were confirmed moving through The Dalles Dam fish ladders during just a five year period (1986-1991 Fish Passage Center data, in: Warren and Beckman, 1993). Furthermore, the pre-dam highly migratory behavoir of white sturgeon, currently exhibited by this species in unimpoundend river systems, does not support the existence of unique populations occuring between all dams throughout the Basin. On the otherhand, the Columbia River Gorge represents a major ichthyofaunal transition zone, an interior populations may share a common ancestry distinct from lower Columbia populations that predates the last glacial era (80,000-10,000 ybp). Thus, a genetically-based defensible definition of evolutionary significant units (populations) is necessary to address these uncertainties. Cost-effective, basin-wide white sturgeon management and conservation mandated in the Council's Fish and Wildlife Program is the framework for the goals and objectives of this project. The project proposed here will advance our basic understanding of the evolutionary and population biology of white sturgeon in the Columbia River Basin and thus allow effective management of genetically defined populations thereby promoting conservation of existing biodiversity.

b. Proposal objectives.

Objective 1. Preliminary assessment of genetic variation among Columbia Basin white Sturgeon based on length variants (VNTRs) of mtDNA.

Objective 2. Assessment of mitochondrial sequence divergence among Columbia Basin white sturgeon.

Objective 3. Assessment of nuclear genetic variation among Columbia Basin white sturgeon.

Information concerning the genetic relatedness of Columbia Basin white sturgeon is the expected outcome of testing the null hypothesis: "All sturgeon populations with in the Columbia Basin form a genetically continous group and are not significantly different." Please see the experimental rationale under Section 7 e for further discussion of objectives/tasks and the rationale for use.

c. Rationale and significance to Regional Programs.

The rationale behind this project is based on important principles of conservation biology. Consistent with FWP objectives, these principles mandate cooperative multi-agency, species-level, research to successfully manage and conserve fragmented populations of widely distributed native fish species, such as white sturgeon. The goal of this project is to establish a critically needed gentic baseline of white sturgeon population structure and definition throughout the entire Columbia Basin using three unique, yet complimentary, genetic analyses (see objectives, Section 7 b). Such a comprehensive baseline is the first required step in defining conservation units for successful management of Columbia River Basin white sturgeon. This definition of conservation units (evolutionary significant units) or populations is also essential for successfully

addressing the nine following FWP objectives (measures): 10.4 (Sturgeon mitigation), 10.4A (Study and evaluate sturgeon populations), five white sturgeon sub-measures with individual agency and tribe responsibilities (10.4A.2, BPA; 10.4A.3, Umatilla Tribe; 10.4A.4, Nez Perce Tribe; 10.4A.5, Spokane and Colville Tribes), and Kootenai River white sturgeon measures: (10.8B.15 and 10.8B16, Kootenai Tribe of Idaho; 10.6C and 10.6C.1, BPA). Furthermore, the following BPA projects depend on this proposal being funded, and vice versa:

BPA Project No.	Title	Affected/Involved Agencies
86-50	White sturgeon Mitigation and Restoration in the Columbia and Snake Rivers Upstream from Bonneville Dam	ODFW, WDFW, USFWS USGS-BRD, NPT, STOI, IPC
94-49	Kootenai River Ecosystem and Fisheries Improvement Study	KTOI
88-64	Kootenai River Fisheries Studies	KTOI
88-65	Kootenai River Fisheries Investigations	IDFG

Rationale for this project is also mandated by the USFWS Darft Recovery Plan for the Kootenai River white sturgeon populations and by the multi-agency (WDFW, CRITFC, ODFW) program entitled "A Review of Alternatives for the Restoration and Management of White Sturgeon Populations and Fisheries on the Columbia between Bonneville and McNary dams (DeVore et al., 1997). The rationale of this draft plan is to "determine if unique stocks (of white sturgeon) exist, and to describe their geographic range. Such stock identification will allow restoration actions to be shaped to ensure genetic diversity is not lost." Such a mandate is consistent with the nine peviously mentioned FWP objectives (measures), and in general, the science of conservation biology. The University of Idaho has developed a favorable working realtionship with all the agencies and tribes mentioned in this proposal, as well as various Canadian fisheries agencies, which is crucial to the successful completion of this project.

The significance of this project to the future of all Columbia River Basin white sturgeon cannot be overemphasized. It is the only project in the entire Columbia Basin that provides the necessary cooperative and comprehensive regional framework within which successful Basin-wide white sturgeon conservation and management is possible.

d. Project history

e. Methods.

Non-lethal tissue samples can be employed with all laboratory procedures in this project. Collection of white sturgeon tissue is a non-invasive, incidental procedure performed during ongoing research when the fish are caught and present no additional

risk to the animal or environment than current agency and tribe research methodology. Participating agencies and tribes have already begun archiving white strugeon tissue samples. We have previously secured or are currently securing receipt of tissue samples to satisfy the requirements for the objectives of this project. We currently have an inventory of 436 tissue samples of white sturgeon from nine of the following 26 populations to be examined:

Sample Code	Sample Location	Subbasin
LCR	Lower Columbia River	Columbia
BP	Bonneville Pool	
TDP	The Dalles Pool	
JDP	John Day Pool	
MCP	McNary Pool	
PRP	Priest Rapids Pool	
WP	Wanapum Pool	
RIP	Rock Island Pool	
CP	Chelan Pool	
WPP	Wells Point Pool	
CJP	Chief Joseph Pool	
LKR	Lake Roosevelt	
KR	Kootenai River	Kootenai/Kootenay
KL	Kootenay Lake	
DL	Duncan Lake	
IHP	Ice Harbor Pool	Snake
LMP	Lower Monumental Pool	
LGO	Little Goose Pool	
LGR	Lower Granite Reservoir	
HCR	Hells Canyon Reservoir	
OXR	Oxbow Reservoir	
BLR	Brownlee Reservoir	
CJS	C.J. Strike Reservoir	
BLR	Bliss Dam Pool	
FSR	Fraser River	Out of Columbia Basin
SAC	Sacramento River	Out of Columbia Basin

Isolated DNA from tissue samples will be amplifed using the polymerase chain reaction (PCR) and nucleotide primers specific for the D-loop region of the mitochondrial genome (mtDNA) (Beckenbach, 1991). The D-loop region contains two areas of interest and each will be examined using different methods. Additionally, nucleotide primers for eight microsatellite loci will also be used with the polymerase chain reaction to amplify the intervening sequences between the primers. This nuclear data will provide genetic information about the nuclear genome in contrast to mitochondrial genome information provided by the other objectives.

Objective 1. Length variation arises in the D-loop of white sturgeon as a consequence of a gain or loss of 1-5 perfectly repeated tandem 78-82 bp sequences (see

Brown et al., 1992, 1996; Buroker et al., 1990). Length variation (or polymorphisms) in the D-loop has been previously examined in a phylogenetic context in white sturgeon of the Columbia Basin (Brown et al., 1992, 1993) but has not been explored as a means of delineating stock structure throughout the Basin. Length variation in amplified mtDNA sequences will be quantified using gel electrophoresis and documented using a computer scanner and image analysis software (SigmaScan/Image). Length variation in amplified D-loop sequences will be examined from as many as 60 individuals per population (provided 60 samples are logistically possible from each population). Thus, allowing 95% confidence limits on the detection of haplotypes that occur in the population at a frequency of 5% or greater in the population under study. Detailed methodologies for this type of analysis is summarized and reviewed by Carvalho and Pitcher (1995) and Avise (1994) and references therein.

Interpopulational differences in mitochondrial D-loop length variation will be assessed, pair-wise, in geographically proximate white sturgeon populations throughout the Columbia Basin these populations include samples from; the Columbia River below Bonneville Dam (LCR), pools and reservoirs behind each Columbia River Dam and each Snake River Dam up to Shoshone Falls, the Kootenai River, Duncan Reservoir, and Kootenai River (24 populations/1440 samples total).

Interpopulational differences in mitochondrial D-loop length variation will be assessed, pair-wise, in geographically separate white sturgeon populations.

Objective 2. An approximate 400 bp segment of the hypervariable, non-repetitive portion of the D-loop region will be sequenced from 10 individuals from each population in Objective 1 to assess the nucleotide divergence in this rapidly evolving portion of the mitochondrial genome (see Appendix A in Section 7 g "References"). For methodologies using sturgeon see Brown et al. (1996), Stabile et al (1996), Miracle and Campton (1995), and Buroker et al. (1990). An automated DNA sequencer and nucleotide primers specific for this region will be used in this task. Samples from white sturgeon populations of the Fraser and Sacramento Rivers will be added to give a geographical perspective to the analysis and an "outgroup(s)" for systematic comparisons ($\leq$120 additional samples).

Objective 3. Nucleotide primer pairs for eight separate microsatellite loci will be used to PCR amplifiy the intervening sequences between primers. All microsatellite primers have been used to previously amplify polymorphic loci in white sturgeon samples (May et al., in press).

Experimental Rationale/Data Analysis: The first objective is intended to provide a rudimentary overall examination of genetic variance among white sturgeon in the Columbia Basin and expedite the dissimination of at least some baseline genetic information for resource and fisheries managers. The first objective is also intended to serve as a rough guide for the two other objectives, essentially a "power test" (see Dizon et al. 1995 for a review) for increasing the confidence of determining of how many samples need to be examined using the other two comprehensive methods. In this context, the scope of the other objectives may change. In Objective 1, frequency differences in length variants between sturgeon populations will be tested (Chi-square

analysis and similar "Goodness of Fit" tests; Zaykin and Pudovkin, 1993). The range of variants has already been surveyed (i.e. the number of classes or families of length variants) as well as the general frequencies of each class, see Brown et al. (1992, 1996). The first objective is simply an extrapolation of this published information to a much larger data set. The second objective will provide information on the rates of nucleotide divergence in the clonaly derived, non-recombinatory, maternally inherited mitochondrial genome. The third objective provides genetic information on the nuclear genome. Specifically, eight separate loci of rapidly evolving non-coding portions of nuclear DNA. Together, the information produced from both Objective 2 and 3 provide separate data sets enabling evaluation of the congruence and dissimilarities of each. This approach provides a statistically powerful evaluation of relatedness among white sturgeon populations.

f. Facilities and equipment.

The Aquaculture Research Institute (ARI) at the University of Idaho directed by Dr. E. Brannon, maintains a fisheries genetics laboratory. This facility has two full time lab technicians, a full time research scientist (Dr. M. Powell), a half time doctoral research assistant (P. Anders), and contains all the equipment necessary to collect, generate, and analyze molecular genetic data necessary for the proposed project. This includes all laboratory equipment, data analysis sofware, office, and clerical support. The University of Idaho's Hagerman Fish Culture Experiment Station (HFCES), with funding from the National Science Foundation (NSF EPSCoR # EPS-9632684), created the Salmonid and Freshwater Fish Research Laboratory. This laboratory is primarily a molecular genetics facility and in conjunction with the ARI fisheries genetics laboratory has already completed preliminary examinations of mitochondrial DNA and karyotypic variation among Kootenai River white sturgeon. Genetic analyses will be divided between the two facilities to expedite the completion of this project. The majority of the nuclear DNA analysis will be conducted at the HFCES Salmonid and Freshwater Fish Research Laboratory. The remaining mitochondrial DNA analysis will be performed at the ARI genetics facility. Dr. D. Campton of the USFWS and a member of the University of Idaho/ARI affiliate faculty will assist with data analysis and interpretation of results.

No field equipment costs or tissue collection is necessary during this project. All tissue samples required have already been collected by coordinating agencies (ODFW, WDFW, IDFG, USGS-BRD, KTOI, NPT, BCMELP, Idaho Power Company, and CSI) or are listed for collection this fiscal year (1998) under their current budgets. Other than personnel and expendible supplies cost, the only requested equipment is a Macintosh-based personal computer and an additional site license for software necessary to analyze microsatellite (multiplex genotyping) data.

The University of Idaho's Aquaculture Research Institute, specifically the fisheries genetics lab, provides a central clearinghouse for systematic and comprehensive

evaluation to establish useful and necessary population genetic data for the benefit of all managers, agencies, and tribes.

g. References.

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Appendix A:

Materials and Methods for direct sequencing of the hypervariable region of the mitochondrial DNA D-loop or control region (Objective 2).

The methods to be employed for this portion of the project are identical to those used recently by one of us (co-P.I. D.E. Campton) to investigate levels of genetic divergence between pallid and shovelnose sturgeon in the Mississippi River drainage (Campton et al., in prep.). These methods, as extracted from the paper of Campton et al. (in prep.), are detailed below (in past tense).

Materials and Methods

Total genomic DNA was extracted from tissue or blood of each specimen by standard phenol/chloroform methods (Sambrook et al. 1989). Extracted DNA was frozen in TE buffer for subsequent use as a template in PCR (polymerase chain reaction) reactions.

DNA primers for amplifying a highly variable region of the mtDNA control region (approximately 500 base pairs) were designed on the basis of published sequences for white sturgeon (*Acipenser transmontanus*), green sturgeon (*A. medirostris*), lake sturgeon (*A. fulvescens*) and Gulf of Mexico sturgeon (*A. oxyrhynchus desotoi)* (Buroker et al. 1990; Brown et al. 1993; Ferguson et al. 1993; Miracle and Campton 1995). These primers were designated L178 (5' AATGTTTCATCTACCATCAAAT) and H701 (5' GGTTTGACAAGAAATATAAGGC 3'), where L and H refer to the light and heavy strands, respectively, and the appended number refers to the 3' base position of the primer with respect to the 1.6 kb reference sequence for white sturgeon (Buroker et al. 1990). The first three bases of the L178 primer overlap with the last three bases of the tandem repeat core sequence described previously for *Acipenser* (Buroker et al. 1990; Miracle and Campton 1995). Consequently, the targeted region for amplification in *Scaphirhynchus* is homologous to the region that is immediately downstream from the region of tandem repeats (VNTR's) in *Acipenser* (see attached Fig. 4 from Campton et al., in prep.).

PCR amplifications followed standard procedures with slight modification. Biotinylated versions of the two primers were used, and an 18-base "universal" M13 sequence was added to the 5' end of the primers to facilitate automated sequencing (see below). Thermal cycling parameters in the PCR reactions were: initial denaturation at 94^{o} C for 3 minutes, followed by 35 cycles of denaturation at 94^{o} C (1 min), annealing of primers at 47^{o} C (1 min), and primer extension at 72^{o} C (1 min). Standard precautions, including negative controls (template-free PCR reactions), were used to test for contamination and to ensure the fidelity of all PCR reactions (Innis et al. 1990).

Streptavidin-coated magnetic beads (Dynabeads M280 streptavidin, Dynal Inc., Sweden) were used to purify PCR products (Mitchell and Merrill 1989). Single-stranded templates were generated by denaturing the magnetically-captured double stranded DNA with fresh 0.15M NaOH. The released (non-biotinylated) strand was then used as a template for the sequencing reactions.

Single stranded sequencing reactions were conducted with fluorescently labelled M13 primers in a robotic work station (Applied Biosystems, Inc., Model 800), and the labelled extension products were analyzed with an automated DNA sequencer (Applied Biosystems, Inc., Model 373A). All sequencing was conducted in the DNA Sequencing Core of the Interdisciplinary Center for Biotechnology Research (ICBR), University of Florida. Initially, both light and heavy strands were sequenced in approximately half of the individuals. Subsequent sequences which exactly matched known haplotypes were collated for analysis whereas new haplotypes were sequenced in the opposite direction to assure the accuracy of haplotype descriptions. Sequences were aligned and edited with SeqEd software (Applied Biosystems, Inc).

Single site substitutions and insertion/deletions (indels) were used to generate a maximum parsimony network among control region haplotypes for *Scaphirhynchus* (Swofford and Olsen 1990). Haplotype frequency differences (a) between pallid and shovelnose sturgeon and (b) between sample sites upstream and downstream of Fort Peck Dam were tested statistically by the randomization chi-square test of Roff and Bentzen (1989) as implemented by Zaykin and Pudovkin (1993).

Control region sequences for pallid, shovelnose, and Alabama sturgeon were compared to the homologous sequences for white and green sturgeon, *Acipenser transmontanus* and *A. medirostris*, respectively (data from Brown et al. 1993). White and green sturgeon are naturally sympatric in coastal drainages of western North America and represent the other genus of North American sturgeon. These latter two species provided a measure by which levels of sequence divergence and evolutionary partitions within *Scaphirhynchus* could be assessed. Five sequences for white sturgeon (CR1, CR2, CR9, CR11, CR19) were selected to reflect the range of nucleotide diversity and genealogical clusters among haplotypes for that species (see Fig. 6 of Brown et al. 1993). The one published sequence for green sturgeon was included in our comparisons. Proportional sequence divergences among haplotypes were estimated by the measure of Tamaru (1992) and compared in a neighbor-joining phylogenetic tree (Saitou and Nei 1987) using the MEGA software package (Kumar et al. 1993). Haplotype and nucleotide diversities were calculated according to equations 8.5 and 10.5, respectively, of Nei (1987).

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Swofford, D.L. and G.J. Olsen. 1990. Phylogeny reconstruction, p.411-501. *In*: D.M. Hillis and C. Moritz [ed.], *Molecular Systematics*. Sinauer Associates, Inc., Sunderland, Massachusetts.

Tamaru, K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition/transversion and G+C content biases. Mol. Biol. Evol. 9: 678-687.

Zaykin, D.V., and A.I. Pudovkin. 1993. Two programs to estimate significance of X^2 values using pseudo-probability tests. J. Heredity 84:152.

Section 8. Relationships to other projects

As previously listed in the table in Section 7.C, several BPA projects depend on this proposal being funded, and vice versa. A partial list of Columbia Basin white sturgeon research includes; population abundance work, stock status determination, early life history research, population recruitment evaluation, harvest management, experimental relocation efforts, potential natural production modeling, and assessment of aquaculture. A common requirement of all these projects is an accurate genetic account or evaulation of Basin-wide white sturgeon populations. This is true because long-term fitness and persistence of fish populations depend their genetic and geographic structuring, and their abilities to adapt to changing environmental conditions based on their genetic variation and phenotypic plasticities. The success of future research and management which may involve conservation or supplementation aquaculture, or relocation, is directly dependent on understanding the genetic structuring within and and among affected or involved populations. The success of recruitment, natural production, and therefore harvest management are also directly dependent on the genetic constitution of pertinent white sturgeon populations. To pursue future management and conservation of white sturgeon without knowing what constitutes separate or distinct populations (ESUs) is an illogical and risk filled approach to managing this recreationally, commercially and spiritually important native species.

Section 9. Key personnel

Name	Employer	Title	FTE/hours
Madison S. Powell	Univ. of Idaho	Principle Investigator	0.5/2080
Ernest L. Brannon	Univ. of Idaho	Co-Principle Investigator	0
Donald E. Campton	USFWS	Co-Principle Investigator	0
Paul J. Anders	Univ. of Idaho	Research Support Scientist	1/2080

Duties for this project and qualifications for the proposed work:

All of the key personnel involved in this project have previously worked with sturgeon genetics. Dr. Brannon has previously published on genetic variaton in white sturgeon using allozyme analysis. Drs. Powell and Brannon are supported with funding from an NSF grant to examine Kootenai River white sturgeon until June 1998. All the procedures to be used in this project are either currently being employed (mitochondrial D-loop variation and sequences variation) or will be employed (microsatellite analysis) by the termination of that contract. This project is simply a greatly expanded version (in sample size and scope) of the current examination of Kootenai River sturgeon. Dr. Campton has previously published on sturgeon genetic variation using essentially the same mitochondrial DNA techniques and is highly competent in the analysis of that type and other types of genetic data. Mr. Anders is currently a Ph.D. student under the direction of Dr. Brannon and laboratory guidance of Dr. Powell and is responsible for a majority of the laboratory work completed under the NSF contract. Mr. Anders, prior to his admission to the University of Idaho, was previously employed as a sturgeon biologist for the Kootenai Tribe of Idaho and the USFWS, and is extremely familiar with white sturgeon biology. Dr. Powell and Mr. Anders will conduct a majority of the laboratory work. Drs. Brannon and Campton will assist in analysis of the data generated and interpretation of the results as they apply to white sturgeon management and conservation.

Curriculum vitae for key personnel follow:

MADISON S. POWELL

Education:
Ph.D., 1995, Texas Tech University
M.S., 1990, University of Idaho
B.S., 1985, University of Idaho

Current employer: University of Idaho, Hagerman Fish Culture Experiment Station
3059 F National Fish Hatchery Road, Hagerman, ID 83332, (208) 837-9096
FAX: (208) 837-6047, email mpowell@northrim.net

Current Responsibilities: Research scientist; supervise fisheries genetics laboratories and lab personnel at the Aquaculture Research Institute and the Hagerman Fish Culture Experiment Station.

Previous employment:

1997-present	Research Scientist, Hagerman Fish Culture Experiment Station, University of Idaho, Hagerman, Idaho
1996-1997	Research Scientist, Aquaculture Research Institute, University of Idaho, Moscow, Idaho
1995-1996	Postdoctoral Fellow, Aquaculture Research Institute, University of Idaho, Moscow, Idaho
1995	Ph.D., Zoology, Texas Tech University
1990	M.S., Zoology, University of Idaho
1985	B.S., Zoology/Biology, University of Idaho

Technical experience:

DNA and RNA isolation, molecular cloning, genomic libraries, DNA fingerprinting, automated sequencing, PCR amplification, RFLP analysis, RAPD analysis, *in vitro* transcription, fluorescence *in situ* hybridization, karyotyping, cell and tissue culture, nucleotide and protein electrophoresis, liquid chromatography, HPLC analysis, small animal surgery, field collection, and identification.

Five publication closely related to this project:

Powell, M.S. G.H. Thorgaard, R.L. Williams, B.A. Robison, J.C. Faler, and E.L. Brannon. Genetic analysis of sockeye salmon (*Oncorhynchus nerka*) in Redfish Lake. Annual Completion Report, U.S. Dept. of Energy, Bonneville Power Administration, Portland. In preparation.

Powell, M.S. and J.C. Faler. Genetic differentiation among early and late spawning populations of kokanee salmon. In preparation, *Can. J. Fish and Aquat. Sci.*

Anders, P., and M. Powell. Karyotypic analysis of an endangered and geographically isolated population of white sturgeon (*Acipenser transmontanus*), In preparation. *Genetica*

Paragamian, V.L., M.S. Powell, J.C. Faler, and S. Snelson. (accepted for publication) Mitochondrial DNA analysis of burbot *Lota lota* stocks in the Kootenai River Basin of British Columbia, Montana, and Idaho. *Trans. Amer. Fish. Soc.*

Baker, R.J., A.D. Simmons, M.S. Powell, J.L. Longmire, and R.D. Bradley. 1996. Utility of a satellite DNA sequence as a genetic marker in a hybrid zone of pocket gophers (Genus Geomys). pp25-34, In: Contributions in Mammalogy: A memorial volume honoring J. Knox Jones Jr. (H.H Genoways and R.J. Baker eds.) Museum of Texas Tech University, Lubbock, Texas.

PAUL J. ANDERS

Education:
Ph.D. Student (7/96 - Present) University of Idaho; Conservation Genetics, white sturgeon
M.S., 1991, Eastern Washington University, Biology, white sturgeon
B.S., 1983, Saint Norbert College

Current employer: University of Idaho, Aquaculture Research Institute, Fish Genetics Lab, Moscow, ID 83844-2260, (208) 885-5830 FAX: (208) 885-5968, email: ande9662@uidaho.edu

Current responsibilities: Oversee and participate in all aspects of mitochondrial DNA analyses of white sturgeon from ID, OR, WA, and BC, Canada. Perform nuclear and mtDNA analyses on salmonid and cyprinid fish species as needed. Prepare scientific reports and manuscripts.

Previous employment:

1996-present:	Ph.D. Research Assistant, University of Idaho, Aquaculture Research Institute, Fish Genetics Lab, Moscow, ID
1993-1996	Fisheries Program Administrator/Fishery Biologist, Kootenai Tribe of Idaho, Bonners Ferry, Idaho
1993	Fishery Biologist, Kootenai Tribe of Idaho, Bonners Ferry, ID
1990-1993	Fishery Biologist U.S. Fish and Wildlife Service, Columbia River Field Station, Cook, WA
1989-1991	M.S. Graduate Research Assistant, Eastern Washington University, Cheney, WA
1987-1990	Fisheries Technician, Idaho Department of Fish and Game, Lewiston, Bonners Ferry, ID

Expertise: I have been professionally involved in research and management of white sturgeon in the Columbia River Basin for 10 years. I have published more than 20 scientific reports and articles on research and management of Columbia River Basin white sturgeon populations, and given dozens of professional presentations of this work. Since 1996, I have been studying conservation genetics and performing genetic analyses of white sturgeon from throughout the Columbia River Basin, in order to develop a basin-wide, species level conservation and management plan for white sturgeon.

Five publications closely related to the proposed project:

Anders, P., and M. Powell. Karyotypic analysis of an endangered and geographically isolated population of white sturgeon (*Acipenser transmontanus*), In preparation.

S. Duke, Anders, P., G. Ennis, R. Hallock, J. Laufle, R. Lauzier, L. Lockard, B. Marotz, V. Paragamian, and R. Westerhof. 1996. White Sturgeon: Kootenai River Population Draft Recovery Plan. Prepared by Region 1, U.S. Fish and Wildlife Service, Portland, OR, USA.

Anders, P. and D. Richards. 1996. Implications of Ecosystem Collapse on White Sturgeon (*Acipenser transmontanus*) in the Kootenai River, Idaho, Montana, and British Columbia. In: Proceedings of the International Congress on the Biology of Fishes, San Francisco State University, CA. July 14-18, 1996, pp. 27-40.

Anders, P. and R. Westerhof. 1996. Conservation Aquaculture of Endangered White Sturgeon (*Acipenser transmontanus*) from the Kootenai River, Idaho. In: Proceedings of the International Congress on the Biology of Fishes, San Francisco State University, CA. July 14-18, 1996, pp. 51-62.

Anders, P.J. 1996, 1995, 1994, 1993. Natural Spawning of White Sturgeon in the Kootenai River. In: Kootenai River White Sturgeon Studies, Annual Reports of Research, Report A. Bonneville Power Administration Project No. 88-64.

ERNEST L. BRANNON

Education:
Ph.D., 1973, Fisheries, University of Washington
B.S., 1959, Fisheries, University of Washington

Current Employer/Responsibilities:
Director, Aquaculture Research Institute, University of Idaho
State Aquaculture Extension Specialists
Professor of Fish and Wildlife Resources
Professor of Animal and Veterinary Sciences

Professional experience:

1988-present:	Director, Aquaculture Institute, University of Idaho, Moscow, Idaho
1984-1988:	Professor, School of Fisheries, College of Ocean and Fisheries Sciences, University of Washington, Seattle
1974-1983:	Director, Finfish Aquaculture Program, College of Fisheries, University of Washington, Seattle, Washington
1973-1975:	Assistant Professor, College of Fisheries, University of Washington, Seattle
1971-1972:	Chief Biologist, International Pacific Salmon Fisheries Commission (IPSFC), New Westminster, B.C., Canada
1969-1971:	Supervisor, Sockeye Management Research, IPSFC, New Westminster, B.C., Canada
1959-1969:	Research Biologist, Fisheries Management, Artificial Propagation, Spawning Channel Development and Fish Culture, IPSFC, New Westminster, B.C., Canada
1953-1959:	Field Management, IPSFC, New Westminster, B.C., Canada

Five publications closely related to the proposed project

Powell, M.S. G.H. Thorgaard, R.L. Williams, B.A. Robison, J.C. Faler, and E.L. Brannon. Genetic analysis of sockeye salmon (*Oncorhynchus nerka*) in Redfish Lake. Annual Completion Report, U.S. Dept. of Energy, Bonneville Power Administration, Portland. In preparation.

Cummings, S.A., E.L. Brannon, K.J. Adams, and G.H. Thorgaard. 1997. Genetic Analysis to Establish Captive Breeding Priorities for Endangered Snake River Sockeye Salmon. Conservation Biology 11(3):662-669.

Brannon, E.L. and A.W. Maki. 1996. The *Exxon Valdez* Oil Spill: Analysis of Impacts on the Prince William Sound Pink Salmon. Reviews in Fisheries Science 4(4):289-337.

Thorgaard, G.H., P. Spruell, S.A. Cummings, A.S. Peek, and E.L. Brannon. 1995. Mixed DNA fingerprint analysis differentiates sockeye salmon populations. *Pages 295-303 in* J.L. Nielsen and D.A. Powers, editors. Evolution and the aquatic ecosystem: Defining unique units in population conservation. Proceedings of the American Fisheries Society symposium 17 (May 23-25, 1994, Monterey, CA).

Brannon, E. and A. Setter. 1992. Movements of white sturgeon in Lake Roosevelt (1988-1991). Final Report, Contract # DE-BI79-89BP7298, Project # 89-44, to the US Department of Energy, Bonneville Power Administration, Division of Fish and Wildlife, Portland, OR. 35 pp.

DONALD E. CAMPTON

Education:
Ph.D., 1986, University of California, Davis, Genetics
M.S., 1981, University of Washington, Seattle, Fisheries
B.S., 1974, University of California, Berkeley, Genetics

Current employer: Abernathy Salmon Technology Center, U.S. Fish & Wildlife Service, 1440 Abernathy Creek Road, Longview, WA 98632, (360) 425-6072, FAX (360) 636-1855, email: Don_Campton@mail.fws.gov

Current responsibilities: Serve as regional fish geneticist and program manager for the U.S. Fish & Wildlife Service on technical matters related to the conservation and management of indigenous fish species and associated fishery resources in the Pacific Northwest including California and Nevada. Genetically characterize hatchery and wild populations, develop regional policies and guidelines to protect genetic resources, establish and maintain information data bases on genetic variation, life history data, and population dynamics of hatchery and wild fish populations.

Professional experience:

1997-present	Fish Geneticist, U.S. Fish & Wildlife Service, Longview, Washington
1992-1997:	Assoc. Prof., Dept. of Fisheries & Aquatic Sciences, Univ. of Florida, Gainesville
1986-1992:	Asst. Prof., Dept. of Fisheries & Aquatic Sciences, U.F.
1981-1986:	Graduate Research/Teaching Assistant, University of California, Davis
1978-1980:	Fishery Research Biologist, Washington Dept. of Game, Olympia, Washington

Expertise: Population and quantitative genetics of fish: molecular methods for studying population structures, evolutionary relationships, and introgressive hybridization; statistical/breeding methods for quantifying genetic variation for quantitative characters, and the effects of hatcheries and artificial propagation on the genetic constitution of hatchery and wild populations of salmonid fishes.

Five publications closely related to the proposed project:

Campton, D.E. 1987. Natural hybridization and introgression in fishes: methods of detection and genetic interpretations, p. 161-192. *IN*: N. Ryman and F. Utter (eds.), *Population Genetics and Fishery Management*, University of Washington Press, Seattle.

Campton, D.E., F.W. Allendorf, R.J. Behnke, and F.M. Utter; M.W. Chilcote, S.A. Leider, and J.J. Loch, 1991. Reproductive success of hatchery and wild steelhead. Trans. Am. Fish. Soc. 120:816-827.

Miracle, A.L., and D.E. Campton. 1995. Tandem repeat sequence variation and length heteroplasmy in the mitochondrial DNA D-loop of the threatened Gulf of Mexico sturgeon, *Acipenser oxyrhynchus desotoi*, J. Heredity 86:22-27.

Campton, D.E. 1995. Genetic effects of hatcheries on wild populations of Pacific salmon and steelhead: What do we really know?, p. 337-353. *IN*: R.G. Piper and H.L. Schramm, Jr. (eds.), *Uses and Effects of Cultured Fishes in Aquatic Ecosystems*, American Fisheries Society, Bethesda, Maryland.

Campton, D.E., A.I. Garcia, B.W. Bowen, and F.A. Chapman. Genetic distinction of pallid and shovelnose sturgeon (*Scaphirhynchus albus* and *S. platorynchus*) based on mitochondrial DNA control region sequences (in prep. for Cons. Biol.)

Section 10. Information/technology transfer

Information generated by this project will be published as peer-reviewed publications and BPA annual reports. Information will also be updated and presented at future Sturgeon Summit Meetings, Kootenai River white sturgeon committee meetings, American Fisheries Society conferences, and BPA project summary conferences. It is critically important for white sturgeon management that information from this project be distributed so that the implications of the results and conclusions can be throughly discussed and reviewed.